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Toxicity of Spinosad to Susceptible and Resistant Strains of House Flies, *Musca domestica*

Jeffrey G. Scott

Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853-0901 USA

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Abstract: The toxicity of spinosad, a new insecticide derived from the bacterium *Saccharopolyspora spinosa*, was evaluated against susceptible and resistant strains of house fly (*Musca domestica* L.). Spinosad was highly toxic to house flies based on 72-h LD_{50} values and the symptoms of poisoning were consistent with a neurotoxic mechanism of action. Spinosad was relatively slow acting, with the maximum toxicity noted at 72 h. Piperonyl butoxide and *S,S,S*,-tributylphosphorotrithioate synergized the toxicity of spinosad by 3·0- and 1·8-fold, respectively, while diethyl maleate had no significant effect. These results suggest that there is a small degree of monooxygenase-mediated spinosad detoxification in house flies, while hydrolases may be only minimally important and glutathione transferases may have no role. There were no substantial levels of cross-resistance detected, except in the LPR strain where a low 4·3-fold cross-resistance was observed. The cyclodiene-resistant OCR strain was 2·7-fold more sensitive to spinosad than the susceptible strain (CS). These results suggest that cross-resistance may not be a limiting factor for the use of spinosad against house flies.

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Key words: Insecta; synergism; neurotoxic insecticide; cross-resistance; spinosad

1 INTRODUCTION

House flies (*Musca domestica* L.) are important vectors of human and animal diseases.[1] Fly control is most commonly achieved with insecticides, but unfortunately, house flies have shown a remarkable ability to evolve resistance to these. This trait, combined with loss of available insecticides through regulatory processes, has resulted in an urgent need for new house fly control agents.

Spinosad is a new and highly promising insecticide, derived from the bacterium *Saccharopolyspora spinosa*, with efficacy against a wide range of insects.[2] However, its effectiveness against house flies has not yet been reported. The mechanism of action of spinosad appears to be unique, with a primary site of attack being the nicotinic acetylcholine receptor and a secondary site of attack possibly being GABA receptors.[3] This unique mechanism(s) of action suggests that resistance due to changes in the target sites of other insecticides (i.e. *kdr* or *Rdl*) would not result in cross-resistance to spinosad. The relative importance of different detoxification pathways for spinosad has also not been reported. Therefore, the synergists piperonyl butoxide (PBO), *S,S,S*,-tributylphosphorotrithioate (DEF) and diethyl maleate (DEM) were used to gain preliminary information on the relative role of cytochrome P450 microsomal monooxygenases, hydrolases and glutathione transferases, respectively, in the metabolism of spinosad.

One concern with all new insecticides is whether or not there will be cross-resistance due to the previous use of other insecticides. To investigate this possibility, the toxicity of spinosad was examined in one susceptible and six insecticide-resistant strains of house fly. These strains of house fly contain one or more mechanisms of resistance, and the combination of these strains represents the major mechanisms of resistance that are known in this species.

E-mail: JGS5@CORNELL.EDU
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2 EXPERIMENTAL

2.1 Insects and chemicals

Seven strains of house fly were used. CS is an insecticide-susceptible strain.[4] LPR is a multi-resistant strain having high levels of resistance to pyrethroid insecticides due to decreased cuticular penetration (*pen*), *kdr* and increased oxidative metabolism[5] mediated by cytochrome $P450_{lpr}$ (i.e. CYP6D1).[6–9] AVER has >1000-fold resistance to abamectin[10] due to decreased penetration and an altered target site.[11] OCR, having high levels of cyclodiene resistance (presumably due to *Rdl*), and Cornell-R, which is highly resistant to organophosphates due to an altered acetylcholinesterase,[12] were provided by Dr F. W. Plapp Jr (Univ. of Arizona). The R12 and R3 strains were isolated through a series of genetic crosses from the LPR strain.[13] R12 is pyrethroid-resistant due to CYP6D1-mediated detoxification, and R3 is pyrethroid-resistant due to *kdr* and *pen*.[5,13] House flies were reared as described previously.[14]

Spinosad was from DowElanco (Indianapolis, IN). PBO and DEM were from Aldrich (Milwaukee, WI). DEF was from Chem Service (West Chester, PA).

2.2 Bioassays

Insecticide (or synergist) was delivered in 0·5 μl acetone to the thoracic notum of female house flies.[15] Synergist was delivered 1 h before the insecticide application at the maximum sublethal dose (3, 10 and 10 μg per fly for PBO, DEF and DEM, respectively). Twenty three- to five-day-old house flies were treated for each dose. A minimum of three doses giving >0% and <100% mortality at 72 h after insecticide treatment was used for each experiment. Each experiment was replicated at least three times. The treated insects were put in 200-ml Sweetheart ice cream cups covered with cheese cloth and held at 25°C. Each cup was provided with a 4-cm dental wick soaked in 15% sugar/water and the dental wick was kept wet during the experiment. Mortality was assessed 72 h after insecticide application. Insects were considered dead if they were on their backs and unable to right themselves when disturbed. Bioassay data were pooled and analyzed based on standard probit analysis[16] as adapted to personal computer use,[17] using Abbott's correction for control mortality.

3 RESULTS AND DISCUSSION

Spinosad is highly toxic to house flies with a 72-h LD_{50} of 24·2 ng per fly to the susceptible CS strain (Table 1). This toxicity is comparable with that of commercially used pyrethroids such as fenvalerate, bifenthrin and permethrin.[5] Spinosad was relatively slow-acting, with LD_{50} values decreasing approximately 2–3-fold between 24 and 72 h (data not shown). Initial symptoms of poisoning included slight hyporesponsiveness and leg tremors leading to ataxia and a rigid paralysis. In some cases, spinosad-treated flies displayed a twitching of their wings and/or protrusion of the ovipositor. These symptoms are consistent with a previous study indicating that spinosad is a neurotoxin.[3] Symptoms of poisoning were similar in all strains.

To evaluate the possible roles of P450 monooxygenases, hydrolases and glutathione transferases in the metabolism of spinosad, the effect of three synergists on the toxicity of the compound to the susceptible strain was examined. PBO caused a 3·0-fold increase in toxicity to the CS strain (LD_{50} = 8·07 ng per fly), suggesting that there is a small degree of monooxygenase-mediated spinosad detoxification in house flies. DEF caused only a 1·8-fold increase in toxicity (LD_{50} = 13·4 ng per fly) while DEM had no significant effect (LD_{50} = 26·9 ng per fly). These results indicate that, in susceptible house flies, hydrolases may be minimally important in spinosad detoxification, and that glutathione transferases may have no role.

To investigate the potential for cross-resistance, the toxicity of spinosad was examined in six strains of house fly variously resistant to other insecticides. LD_{50}

TABLE 1
Toxicity of Spinosad to Seven Strains of House Fly Treated by Topical Application

Strain	*72-h LD_{50} (ng per fly) (95% CI)*	*Slope (SE)*	*n*	*RR*[a]
CS	24·2 (19·2–29·2)	2·8 (0·3)	920	—
Cornell-R	21·0 (17·9–24·2)	1·9 (0·2)	560	0·9
AVER	46·8 (36·5–58·0)	2·1 (0·2)	300	1·9
LPR	103 (88·5–119)	1·7 (0·2)	420	4·3
OCR	8·90 (7·75–10·2)	2·1 (0·2)	420	0·4
R12	44·6 (26·6–60·5)	2·9 (0·4)	420	1·8
R3	33·1 (29·6–37·7)	2·6 (0·2)	360	1·4

[a] RR = Resistance Ratio (LD_{50} of resistant strain/LD_{50} of CS susceptible strain).

values between susceptible populations (i.e. strains) can vary somewhat. These relatively small differences are best referred to as sensitive or tolerant populations, with the term cross-resistance being reserved for cases where resistance to one insecticide gives rise to a population that is resistant to another insecticide.[19] If the parental population (i.e. prior to selection) is not available for comparison it may be difficult (when resistance ratios are small) to differentiate between tolerance and cross-resistance (or between sensitivity and negative cross-resistance). Based upon experience with bioassays on house flies, the term cross-resistance is best reserved for cases where resistance ratios are $>4{\cdot}0$. Based on this criterion there were no substantial levels of cross-resistance detected, except in the LPR strain where a low, 4·3-fold, cross-resistance was observed. Given that there was no cross-resistance in the R12 or R3 strains, it appears that the low level of cross-resistance in LPR is not due solely to CYP6D1-mediated detoxification, *kdr* or *pen*. The OCR strain was more sensitive to spinosad than the susceptible strain by 2·7-fold. These results indicate that, at least in house flies, the mechanisms responsible for resistance to the major classes of insecticides do not confer levels of cross-resistance that would be likely to present any limitations on the use of this promising new insecticide.

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